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Epidemiological Studies of Asthma and Neurodevelopmental Disorders in Children

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Epidemiological Studies of Asthma and Neurodevelopmental Disorders in Children

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To my family and Catarina

ABSTRACT

Asthma and neurodevelopmental disorders including autism spectrum disorders (ASD) and attention deficit hyperactivity disorders (ADHD) are common diseases starting in early childhood. The prevalence of both diseases is rising and little is known about the potential genetic and environmental risk factors. Therefore, the overall aim of this thesis was to investigate the early life risk factors associated with the subsequent development of asthma and neurodevelopmental disorders, especially ASD using population- and family-based designs.

In Study I, we investigated the association between parental socioeconomic status (measured by income and education), risk of asthma, and patterns of medication dispenses in a large population-based cohort of preschool children. We found an age-varying effect on the risk of asthma, but no effect on the pattern of medication dispenses by parental income. Parental education, however, was negatively associated with asthma regardless of age and positively associated with controller medication dispenses.

In Studies II and III, we evaluated the association between exposure to traffic-related air pollution and neurodevelopmental disorders among children born in Stockholm during 1992-2007. In contrast to previous findings, there was no clear association between air pollution during pregnancy or early infancy and subsequent risk of ASD and ADHD. Residual confounding from parental socioeconomic status and psychiatric diagnoses can partly explain the findings and the differences observed in some subgroups.

In Study IV, we assessed the association between parental asthma, use of asthma medication during pregnancy and the risk of ASD in offspring by comparing cases and controls in the general population and within families. Maternal, but not paternal, asthma, was associated with a slightly increased risk of ASD, which was neither confounded by familial factors shared among half-siblings and cousins nor mediated through use of asthma medications during pregnancy.

In conclusion, these collective studies shed light on the relationship between many early life risk factors and subsequent risk of asthma and neurodevelopmental disorders. The higher risk of incident asthma and lower rate of controller medication dispenses among young children with lower parental socioeconomic background warrants clinical attention. Traffic-related air pollution, despite being a major concern to the general public, was not associated with ASD and ADHD in the Swedish urban setting. Furthermore, the association between maternal asthma and offspring ASD appeared to be persistent, suggesting the importance of future investigation into potential biological mechanisms.

LIST OF SCIENTIFIC PAPERS

I. **Gong T**, Lundholm C, Rejno G, Mood C, Langstrom N, Almqvist C. Parental socioeconomic status, childhood asthma and medication use - a population-based study. PLoS One. 2014;9(9):e106579.

II. **Gong T**, Almqvist C, Bolte S, Lichtenstein P, Anckarsater H, Lind T, Lundholm C, Pershagen G. Exposure to air pollution from traffic and neurodevelopmental disorders in Swedish twins. Twin Res Hum Genet. 2014;17(6):553-62.

III. **Gong T**, Dalman C, Wicks S, Dal H, Magnusson C, Lundholm C, Almqvist C, Pershagen G. Perinatal exposure to traffic-related air pollution and autism spectrum disorders. (Submitted)

IV. **Gong T**, Lundholm C, Rejnö G, Bölte S, Larsson H, D'Onofrio B, Lichtenstein P, Almqvist C. Parental asthma and maternal asthma medication during pregnancy and risk of offspring autism spectrum disorder. (Submitted)

Related publications *(not included in thesis)*

I. Rejno G, Lundholm C, **Gong T**, Larsson K, Saltvedt S, Almqvist C. Asthma during pregnancy in a population-based study--pregnancy complications and adverse perinatal outcomes. PLoS One. 2014;9(8):e104755.

II. Khashan AS, Kenny LC, Lundholm C, Kearney PM, **Gong T**, Almqvist C. Mode of obstetrical delivery and type 1 diabetes: a sibling design study. Pediatrics. 2014;134(3):e806-13.

III. Almqvist C, Ortqvist A, **Gong T**, Wallas A, Ahlen K, Ye W, Lundholm C. Individual maternal and child exposure to antibiotics in hospital - a national population-based validation study. Acta Paediatrica. 2015 Apr;104(4):392-5.

IV. Guxens M, Ghassabian A, **Gong T**, Garcia-Esteban R, Porta D, Giorgis-Allemand L, Almqvist C, Aranbarri A, Beelen R, Badaloni C, Cesaroni G, de Nazelle A, Estarlich M, Forastiere F, Forns J, Gehring U, Ibarluzea J, Jaddoe VW, Korek M, Lichtenstein P, Nieuwenhuijsen MJ, Rebagliato M, Slama R, Tiemeier H, Verhulst FC, Volk HE, Pershagen G, Brunekreef B, Sunyer J. Air pollution exposure during pregnancy and childhood autistic traits in four European population-based cohort studies-The ESCAPE Project. Environmental Health Perspectives. 2016 Jan;124(1):133-40.

CONTENTS

LIST OF ABBREVIATIONS

ADHD	Attention Deficit Hyperactivity Disorder
ASD	Autism Spectrum Disorder
A-TAC	Autism-Tics, ADHD and other Comorbidities inventory
ATC	Anatomic Therapeutic Chemical
BMI	Body Mass Index
CATSS	Child and Adolescent Twin Study in Sweden
CDR	Cause of Death Register
CI	Confidence Interval
DAG	Directed Acyclic Graph
DSM	Diagnostic and Statistical Manual of Mental Disorders
HAB	Habilitation Register
HR	Hazard Ratio
ICD	International Classification of Diseases
ICS	Inhaled Corticosteroid
ID	Intellectual Disability
IgE	Immunoglobulin E
LABA	Long-acting β2-agonist
LISA	Longitudinal Integration Database for Health Insurance and Labor Market Studies
LTRA	Leukotriene Receptor Antagonist
MBR	Medical Birth Register
MGR	Multi-Generation Register
NDD	Neurodevelopmental Disorder
NPR	National Patient Register
OR	Odds Ratio
PASTILL	Clinical Database for Child and Adolescent Psychiatry in Stockholm
PDR	Prescribed Drug Register
PIN	Personal Identification Number
PM	Particulate Matter

RCT	Randomized Controlled Trial
SABA	Short-acting β2-agonist
SES	Socioeconomic Status
SYC	Stockholm Youth Cohort
TPR	Total Population Register
VAL	Stockholm Regional Health Care Data Warehouse

1 Background

1.1 Asthma

1.1.1 Disease characteristics

The word ‘asthma’ (originally from Greek) appeared first in the *Corpus Hippocraticum* as a medical word indicating difficulty breathing or shortness of breath. Today, these are referred to asthma-like symptoms. Asthma is currently defined as “a heterogeneous disease, usually characterized by chronic airway inflammation” (1).

The heterogeneity of asthma means that: 1) there is great variation in clinical and physiological features; 2) there is no single pathway to disease development (see *1.1.4 Etiology and risk factors*); and 3) there is no common therapy suitable for all patients (see *1.1.3 Diagnosis and treatment*).

Symptoms of childhood asthma include wheezing, cough, shortness of breath and chest tightness (2). Distinct ‘subgroups’ (phenotypes) of asthma with certain observable characteristics have been identified. However, there is a lack of a common phenotypic definition to sub-categorize asthma patients (3). For example, asthma can be defined as early-onset/transient, late-onset, persistent (based on the age of onset of wheezing), eosinophil or non-eosinophil (based on the presence of airway inflammatory markers), and atopic or non-atopic (based on the immunoglobulin E [IgE] antibody responses and co-existing allergic diseases) among others (3). Since phenotypes are not mutually exclusive, i.e. a patient can belong to more than one asthma phenotype, it is challenging to interpret and compare research findings.

1.1.2 Prevalence

Asthma is one of the most common chronic diseases among children (1). Globally, the prevalence of childhood asthma has varied over the past few decades, with some countries reaching a plateau or decline in prevalence after decades of increase (see Figure 1) (4-7). There is no evidence of a global decline (8). For example, in high-income countries with high asthma prevalence, such as the U.K., the U.S., Australia, and New Zealand, plateaus or slight decreases in prevalence have been observed. Among Swedish children, asthma prevalence is now stabilized at around 10% (9). In low- and middle-income countries in Africa and Latin America, asthma prevalence is lower, but a recent increasing trend has been reported. (4). The genetic variations (i.e. heritability) do not seem to simply account for the differences in asthma prevalence over time by countries. The role of the environment, possibly associated with economic growth, seems to be increasingly meaningful to the development childhood asthma.

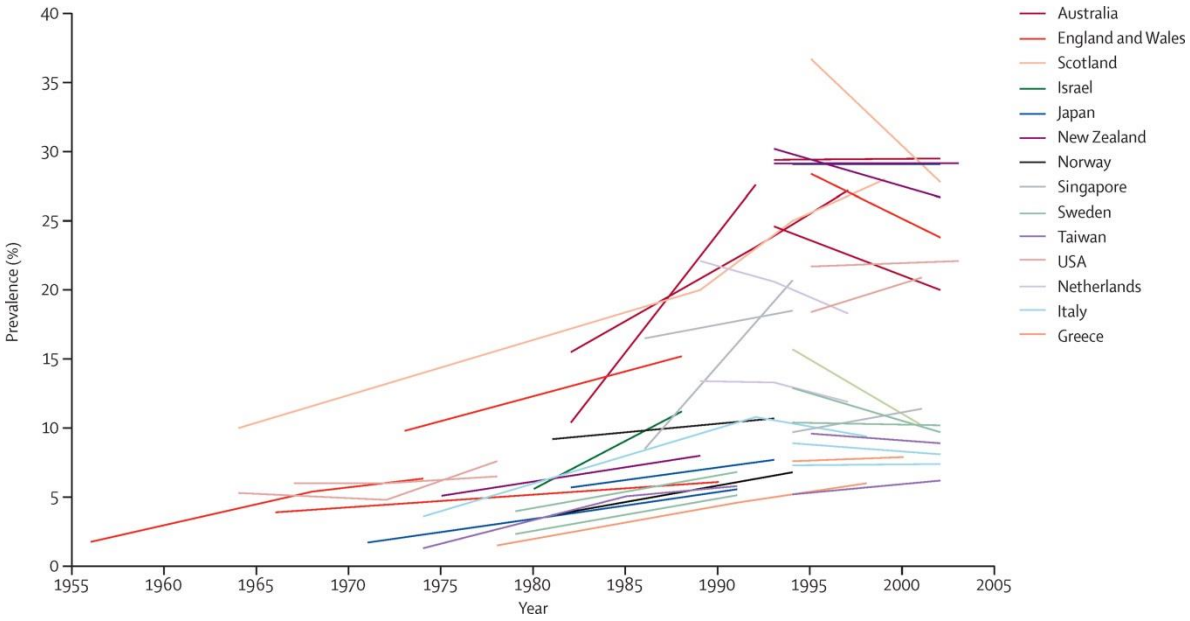


Figure 1. Global trends in prevalence of childhood asthma symptoms by country. From Beasley R, et al. Lancet. 2015 Sep 12;386(9998):1075-85. Reproduced with permission from Elsevier.

1.1.3 Diagnosis and treatment

The diagnosis of asthma is based on the patient's history of respiratory symptoms, family history of asthma or other allergic diseases, physical examination, and diagnostic tests including breathing tests for lung function, airway inflammation and responsiveness, as well as tests for allergic sensitization. Clinical, pathological and physiological features can vary from patient to patient and none of these signs are mandatory for the diagnosis (2). Asthma, especially in children under 5 years of age, can be difficult to diagnose because their wheezing may be temporary and attributable to colds or other respiratory infections. Therefore, prescribing some medications to test the symptom improvement and identifying risk factors for asthma and co-existing allergies can be helpful before making the actual asthma diagnosis for this age group (1).

To better manage patients with asthma in a long-term perspective, pharmacological and non-pharmacological treatments should be involved and follow-up assessment of asthma control and exacerbations is important. Pharmacological treatments for asthma include controller medications (i.e. inhaled corticosteroids [ICS], leukotriene antagonists [LTRA]), symptom-reliever medications (i.e. short-acting β_2-adrenoceptor agonists [SABA or β_2-agonists]), and other add-on medications (i.e. long-acting β_2-adrenoceptor agonists [LABA], anti-IgE monoclonal antibody) for patients with severe persistent asthma. For example, the Swedish Pediatric Society recommends a step-wise treatment algorithm for children under 5 years of based on the severity of individual symptoms and response to treatment (see Figure 2) (10). Non-pharmacological interventions often include prevention and avoidance of risk factors such as tobacco smoke and indoor allergens, as well as breathing exercises (1, 11, 12).

Monitoring tools including asthma control questionnaires and asthma control tests can also be used to help clinicians and patients reach the goal of treatment (13).

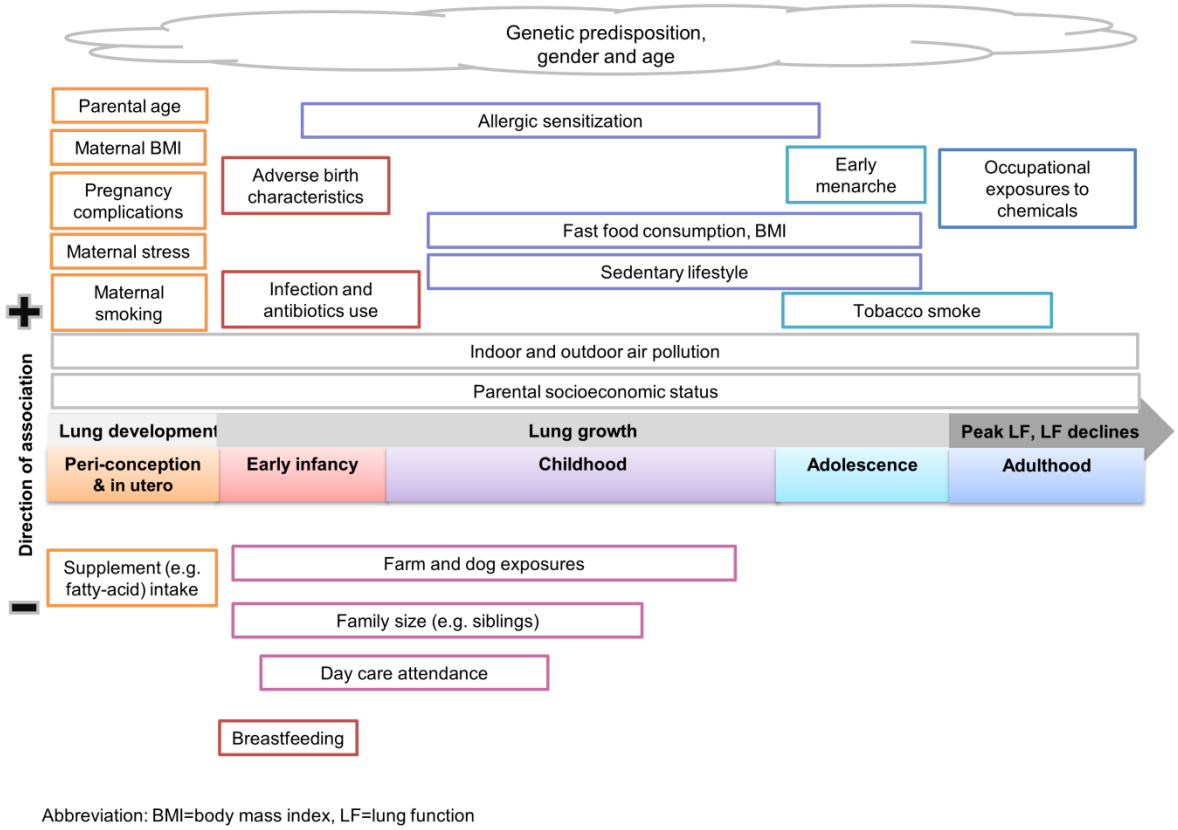
Step 1a	Step 1b	Step 2	Step 3	Step 4
SABA when needed				
	Low dose of Fluticasone / LTRA periodically	Low-to-moderate dose of ICS / LTRA continuously	Low-to-moderate dose of ICS +LTRA / LABA*	Moderate-to-high dose of ICS +LTRA+LABA*

*LABA should be given only to children above 4 years of age.

Figure 2. Stepwise approach for treating asthma in children under 5 years of age. Modified from the guideline for maintenance treatment among children by the Swedish Pediatric Society's section of allergy (10). ICS=inhaled corticosteroids; LABA=long-acting β_2 agonists; LTRA=leukotriene antagonists; SABA=short-acting β_2 agonists;

1.1.4 Etiology and risk factors

Asthma is a disease with multi-factorial etiology. The genetic factors (heritability) explain more than 50% of individual variations in the liability to asthma in the general population (14). A recent consortium-based genome-wide association study identified several loci on chromosomes 2, 3, 6, 9, 15 and 22 that were associated with asthma at all ages (15). However, these could not contribute to all incident cases over the past few decades and explain all the variation by geographic locations mentioned previously. A growing body of evidence suggests both perinatal and early life environmental factors may play a significant role for the prevalence of asthma (summarized in Figure 3) (5, 16). For example, perinatal factors including parental age, maternal body mass index (BMI), maternal stress, smoking and diet during pregnancy, and adverse birth characteristics such as low birth weight have been found to be associated with asthma (17-22). Early life infections, exposure to tobacco smoke, air pollution, and other allergens have all been linked to increased risk of asthma in childhood and adolescence, whereas dog and farm exposure were found to be negatively associated with asthma (20, 23-28).



Abbreviation: BMI=body mass index, LF=lung function

Figure 3. Risk factors for childhood asthma at a glance

Socioeconomic status

The association between socioeconomic status (SES) and diverse adverse health outcomes has been known for decades (29). However, three main issues remain in the research topic of SES and asthma in terms of the measurement of SES, plausible explanations and mechanisms behind the association, and differences in healthcare service utilization.

1.1.4.1 Defining and measuring SES

SES, sometimes referred as "socioeconomic position", is commonly defined as a combined measure of the social status and economic situation of an individual or group. According to a recent systematic review, the measurement of SES (or SES proxies) differs widely between studies depending on data availability, and geographic and cultural differences (30). For example, family income or highest education level attained within the household are commonly used measures in studies from Australia, Brazil, Canada, the Netherlands, the U.K., and the U.S. (31-35). Studies from European countries often use occupational classifications to measure individual SES (36-39). For example, in a previous study from Sweden there was a lower rate of asthma, rhinitis, and IgE sensitization in children whose parents had white-collar work compared to those whose parents had blue-collar work (37). The type of enrolled medical insurance, frequently used in American studies, is a measure which is less often reported from countries with universal health care systems (31, 40). Area-level and country-level SES measures, for example area deprivation, average household

income, or national gross national income, have also been used in previous studies to investigate potential effects of SES on asthma (34, 41, 42). In addition to differing measurements for SES, a selected measure of SES may vary over time. For example, education measures tend to be stable, whereas income can be volatile and fluctuate considerably during the life-course of an individual or within a family. The temporal changes of income are difficult to capture and have not been studied in terms of asthma incidence among young children. Taken together, it is often difficult to make direct comparisons of findings between studies.

1.1.4.2 Explanations on the association between parental SES and childhood asthma

Of the many SES-associated environmental risk factors for asthma, the hygiene hypothesis summarizes some of the key risk factors. This hypothesis attributes the increased incidence of asthma and allergic diseases to improved hygiene level, smaller family size, and less exposure to microbe-enriched environments during early-life (43, 44). This hypothesis was investigated broadly in epidemiological studies and used to explain the patterns of SES and childhood asthma prevalence at country level (45). However, it has been challenged recently due to an increasing body of evidence (46). For example, the observed decrease or plateau in asthma prevalence that has been observed in some cohorts was not seen in other allergic diseases including rhinitis and food allergy (47, 48). Children from minority ethnic backgrounds in the U.S. and other socioeconomically disadvantaged families in the U.K. were found to have higher asthma risk (49-51). Income and education measures may act as proxies for certain lifestyle or behavioral factors in these studies. For example, increases in exposure to allergens and psychological distress, as well as decreases in access to healthcare and breastfeeding are all correlated with lower parental income, and have been proposed to explain the observed association between SES and asthma occurrence and morbidity (5). However, it is still difficult to disentangle how measures of lifestyle and behavior factors affect SES because they are often correlated with each other, and all information is not available on a population level.

1.1.4.3 Variations in healthcare service utilization on a national level

As well as being a risk factor for the occurrence of asthma in general population, SES may be related to health disparities observed among asthmatic patients (52). On one hand, a significant amount of research has shown that SES is associated with differences in healthcare service utilization. Several studies from the U.S. have indicated that children with low-income or minority family backgrounds received less asthma specialist care, but more emergency department visits and hospitalizations (53, 54). A study from an urban setting in Canada, which has a universal healthcare system, reported that two-thirds of asthmatic children did not receive follow-up care after an emergency department visit (55). On the other hand, the amount of emergency and inpatient care can be reduced by improved adherence to asthma treatment (56, 57). However, these findings were restricted to rather short observational period. The measurement of healthcare utilizations related to preschool asthma under a universal healthcare system has not been investigated on national level either.

In summary, further evidence on parental SES and childhood asthma is still needed, as is a standard SES metric. Whether there is a difference in asthma-related healthcare utilization in a universal healthcare system also remains unclear.

1.2 Neurodevelopmental disorders (NDDs)

Neurodevelopmental disorders (NDDs) are early onset conditions of behavioral and cognitive impairment associated with the maturation and architecture of the central nervous system (58). Individuals with NDDs may suffer from mild to severe alterations in general intellectual abilities, motor skills, learning, memory, social cognition, executive functioning, language and speech. Some NDDs have multifactorial etiologies and affect more males than females (59). Examples of commonly researched disorders include autism spectrum disorder (ASD), intellectual disabilities (ID), attention deficit hyperactivity disorder (ADHD). Comorbidity with other NDDs is also common (58).

1.2.1 Autism spectrum disorder (ASD)

1.2.1.1 Clinical characteristics

ASD is a concept uniting the formerly separate diagnoses of autistic disorder, Asperger disorder, and pervasive developmental disorder-not otherwise specified (58, 60). ASD is characterized by social communication and interaction difficulties, alongside restricted interests and repetitive behaviors causing impairment in adaptive functioning (61, 62). ASD is a life-long neurodevelopmental condition and early signs usually present between 18 and 24 months of age (63, 64). ASD co-occurs with ID, ADHD, social anxiety disorder, as well as somatic conditions including epilepsy, sleep disturbances, immune dysregulations, and gastrointestinal symptoms (i.e. diarrhea, constipation, and abdominal pain) (65-69).

1.2.1.2 Prevalence

Autism was long considered a rare condition and until the end of the 1990s only a few studies reported prevalence above 0.5% (70). However, since the year 2000, prevalence estimates and diagnoses rates have been rising. A prevalence of at least 1% in the general population is currently widely accepted, but some studies report rates of 2-3% (71-73). The development of a broader classification of the autism spectrum, growing awareness and knowledge among health professionals and rising public concerns have all contributed to this rise in prevalence. Still a certain "true" increase of ASD due to environmental changes cannot be excluded (74).

Unlike asthma, no geographic variation has been reported for the prevalence of ASD (75). Population-based studies in the U.K., the U.S., Sweden, Japan and Korea reported ASD prevalence between 1-2% among children (71, 72, 76-78). By contrast, very few prevalence studies have been conducted in low- and middle-income countries (75). Furthermore, a strong gender bias in ASD prevalence towards males (approximately 4:1) is observed with striking consistency.

1.2.1.3 Etiology and risk factors

The etiology of ASD is unclear (79). Approximately 10% of cases of ASD are attributable to known genetic causes, such as Down's syndrome and fragile X syndrome. Other genetic and non-genetic (environmental) risk factors, as well as interactions between genes and environments are thought to explain the majority of cases (80).

The genetic perspective

Earlier twin and family studies suggested a high (≥70%) heritability of ASD (81-83). However, recent findings suggest that the heritability might be at around 50% and that environmental components play a substantial role (84-86). Previous genome-wide association studies have identified many loci of small effect, but study sample sizes were limited (87, 88). Some other studies have also demonstrated copy number variations and *de novo* mutations as important genetic components for ASD(89). The majority of these mutations were explained by advanced paternal age (90)

The environmental perspective

A variety of environmental risk factors for ASD comprising pre-, peri- and post-natal exposures to infections, chemical substances, developmental characteristics, and other social-demographic influences have been examined in the past decades and contributed to the field progress (See Table 1). On one hand, some environmental exposures (e.g. infections) may alter the development of the immune system and the central nervous system and be subsequently associated with a broader range of neurodevelopmental deficits (69, 91, 92). On the other hand, inconsistencies and controversial findings are also reported concerning the role of some environmental factors such as ambient air pollution in the etiology of ASD. Exposure to air pollution, known to be a major public health concern, often varies by ethnicity and other measurements of SES (93). In some metropolitan cities in the U.S., households with lower SES are more likely to reside in inner-city locations where lower quality housing services (i.e. physical structure of the building, neighborhoods, and infrastructure) are offered. However, in Stockholm, people with lower SES tend to live in suburbs and are less frequently exposed to traffic-related air pollution than those with higher SES (94). Therefore, the way of characterizing SES deserves deliberate consideration and may act as a potential source of imprecisely-adjusted confounders (often referred to as residual confounding) for the potential association between ambient air pollution and ASD.

1.2.1.4 Air pollution as a risk factor for ASD

Exposure to ambient air pollution has been related to a variety of adverse health outcomes, primarily diseases of the cardiovascular and respiratory systems (95). Ambient air pollutants consists of a mixture of different components from different sources, some commonly used markers include particulate matter (PM), nitrogen oxides (NO_x), ozone (O_3), carbon monoxide (CO) and sulfur dioxide (SO_2). PM is a mixture of solid and liquid particles suspended in air, some of which are also inhalable and could induce physiological and

Table 1. A list of identified environmental risk factors for ASD with estimated associations.

Factors	Association
Socio-demographic, developmental, lifestyle	
Gender – male	Positive (96)
Season of conception	Various (97, 98)
Advanced parental age	Positive (99, 100)
Parental alcohol and drug addiction/misuse	Positive (101)
Parental ethnicity and migration	Inconclusive (102-104)
Maternal obesity	Positive (105)
Urbanicity	Positive (106)
SES	Inconclusive (65, 107-109)
Restricted fetal growth, and other birth characteristics	Positive (110)
Infections	
Maternal infection during pregnancy	Positive (111, 112)
Early childhood infection	Positive (113)
Parental medical conditions	
Parental auto-immune disorders	Positive (114, 115)
Pregnancy complications (not specific to one)	Positive (116)
Maternal asthma and allergies	Inconclusive (115, 117, 118)
Medication use	
Antidepressant	Inconclusive (119-121)
Asthma medications (terbutaline here)	Positive (122)
Pollutants-related	
Ambient air pollution	Inconclusive (123-127)
Pesticides	Positive (128, 129)
PVC flooring	Positive (130)
Diet and nutrients	
Maternal vitamin D intake	Negative (131, 132)
Maternal fatty acid intake	Negative (133)
Maternal folic acid intake	Negative (134, 135)

pathological responses (136). Common indicators for PM usually refer to particles with diameters <10 μm (PM_{10}), with diameters between 2.5 and 10 μm (PM_{coarse}), fine particles with diameters <2.5 μm ($PM_{2.5}$), and ultrafine particles with diameters <0.1 μm ($PM_{0.1}$) based on sizes of particles. Local PM_{10} in Stockholm primarily originates from road dust related to road traffic but the influence of long range transport is also substantial. The concentrations of PM_{10} can vary by season depending on meteorological conditions. The urban background level for PM_{10} has dropped 10% since mid of 1990s. However, many streets within the Stockholm city still have higher pollutant levels than current air quality guidelines (20 $\mu g/m^3$) (137, 138). NO_x consists mainly of nitrogen monoxide (NO) and nitrogen dioxide (NO_2), and

is often used as a marker of the exhaust originating from road traffic. The proportion of NO and NO_2 in the air depends on the intensity of sunshine and the levels of ozone.

Exposure to air pollution in urban areas has been associated with several adverse health effects in children, including asthma, allergies and lung function disturbances (139, 140). Recent studies have investigated the role of ambient air pollutants including O_3, CO, NO_x, SO_2, PM, metals, and other hazardous air pollutants on the development of ASD (Table 2). All studies considered exposure during the perinatal period, and ASD were assessed from doctor diagnoses, and/or questionnaire-based autistic traits. Windham and colleagues from California, the U. S. first reported a positive association between prenatal exposure to PM_{10} and ASD (141), which has been replicated in further studies from North Carolina, the U.S., and Taiwan (123, 126, 142, 143). However, Raz et al. did not find any PM_{10}-associated ASD risk, but a positive association between $PM_{2.5}$ and ASD (126). Guxens et al. did not find any association of PM_{10}, PM_{coarse}, or$PM_{2.5}$ and autistic traits (125). Three studies have also observed a trimester-specific effect of PM_{10} (123, 126, 127). For example, a 34-40% increased risk of ASD was observed in one study with a higher PM_{10} level during 3rd trimester, after adjustment for other exposure periods (123). Only one study has investigated the effect on NO_x with null findings (125), but three other studies reported NO_2-associated ASD risks (124, 127, 142). To sum up, the evidence linking ambient air pollution exposure to ASD is not consistent and the role of residual confounding, certain pollutants, and specific timing of exposure should be further explored.

Table 2. Short summary on different types of pollutants from recent publications on ASD and air pollution.

Studies \ Pollutants	**O_3**	**CO**	**NO_x**	**SO_2**	**PM**	**Metals***	**HAP#**
Windham GC et al, 2006 (141)					Yes	Yes	Yes
Palmer RF et al, 2009 (142)						Yes	
Kalkbrenner AE et al, 2010 (143)					Yes	Yes	Yes
Volk HE et al, 2013 (127)	Yes		Yes		Yes		
Becerra TA et al, 2013 (124)	Yes	Yes	Yes		Yes		
Jung CR et al, 2013 (144)	Yes	Yes	Yes	Yes	Yes		
Kalkbrenner AE et al, 2015 (123)					Yes		
Raz R et al, 2015 (126)					Yes		
Guxens M et al, 2016 (125)			Yes		Yes		

Note: O_3 = ozone, CO = carbon monoxide, NO_x = nitrogen oxides, SO_2 = sulfur dioxide, and PM = particulate matter. *Metals include antimony, beryllium, cadmium, chromium, lead, manganese, mercury, and nickel. # Hazardous air pollutants (i.e. HAPs) include hazardous air pollutants listed on Environmental Protection Agency's air toxics website.

1.2.1.5 Parental asthma as a risk factor for ASD

An increasing body of literature has described the potential role of dysregulated immune systems and activated inflammation pathways in the central nervous system among patients with ASD (69, 91). For example, several studies have described abnormal B-cell, T-cell, and NK-cell functions, elevated cytokine responses, decreased immunoglobulin levels, and increased autoantibody production among individuals with ASD (145-149). Other studies have also proposed that asthma and other immune-mediated diseases may be comorbid with ASD (150-152) , or have a higher prevalence among family members of children with ASD (117, 153, 154). However, this evidence was based on relatively small clinical samples and systematic investigations of the common etiology of immune-mediated diseases and ASD are still needed to provide a better understanding of autism.

Parental asthma, especially maternal asthma which also complicates pregnancy, has been associated with poor birth characteristics including smaller size for gestational age, low birth weight, and congenital malformations (155). Very few studies have addressed the long-term consequences in offspring, for example the occurrence of ASD in offspring born to parents with asthma (114, 115, 117, 118, 156, 157). Two studies have indicated that maternal asthma may be a risk factor for ASD (114, 117), the rest have reported an association with only one subtype of ASD (118) or no association (115). However, there is limited evidence to suggest that paternal asthma is not associated with ASD risk (130, 156).

There are two possible hypotheses for the association between parental asthma and offspring ASD. First, the association could be due to shared environmental and/or genetic factors (i.e. familial aggregation of asthma and ASD) within the family. For example, shared environmental factors including maternal nutrient intake (133, 158-162), infections (111, 163), as well as other pregnancy and delivery complications (164-167) have been linked to maternal asthma and offspring ASD. Shared genetic factors could be the explanation if the effects of maternal and paternal asthma on ASD risk are equivalent. Second, the association may be due to unique environmental exposures related to asthma, such as asthma medication use during pregnancy. Previous studies have suggested a link between in utero exposure to β2-agonists, ASD and other developmental disorders in offspring (122, 168, 169). This may be caused by over-stimulating the β2-adrenergic receptor during gestation and altering the fetal neurodevelopment (170, 171). Thus, there is reason to believe that familial aggregation of asthma and ASD can be attributed to maternal use of β2-agonists during pregnancy.

1.2.1 Attention Deficit Hyperactivity Disorder (ADHD)

ADHD is one of the most common NDDs and often characterized by symptoms of inattention, and hyperactivity/impulsivity and with an age-of-onset prior to 12 years, according to the Diagnostic Manual of Mental Disorders, 5th edition (DSM-5) classification (172). The prevalence of ADHD is around 5% in children worldwide (with male predominantly) and does not seem to vary by locations (173). Like other complex disorders, the etiology of ADHD is not clear. Genetic, early environmental factors and gene-

environmental interaction can possibly contribute to the ADHD risk. The comorbidity with other NDDs such as ASD, motor disorders, and other behavioral problems is frequent (174).

1.2.2 Intellectual Disability (ID)

ID is characterized by low general intellectual capacities (IQ < 70) causing significant difficulties in adaptive functioning (175). The diagnoses of ID based on ICD-10 and DSM-IV classification systems are widely used and the levels of severity are dependent on the IQ score (175, 176). Approximately 0.5 to 3% of children are affected by ID, but estimates vary by country, study design, age, gender, severity, and parental SES (177, 178). In terms of etiology, genetic factors play an important role in ID, but recent studies have found pre- and peri-natal factors as well as other environmental exposures can also be linked to increased risk of ID (179, 180). Furthermore, ID can occur with or without congenital malformations, neurological disorders such as epilepsy, or other NDDs including ASD and ADHD.

2 Aims

This thesis explores the contribution of population and family studies to the understanding of the etiology of asthma and ASD.

In particular, we aim:

- to explore the association of parental SES and the risk of asthma, as well as the patterns of medication intake in a nationwide register-based cohort of preschool children (**Study I**);

- to explore the risk of ASD in relation to traffic-related air pollution exposure during fetal and early life (**Studies II & III**);

- to investigate the association between parental asthma, maternal asthma medication and risk of ASD in offspring, and whether it is confounded by familial factors (**Study IV)**;

3 Materials and Methods

All data obtained from registers and used in all studies are discussed in this chapter. Sweden and the other Nordic countries offer unique opportunities for population-based observational researches using the rich and high-quality register-based data. The register data include individual information from pregnancy and birth, to disease onset such as asthma, migration, and death and can be retrieved prospectively or retrospectively.

3.1 Register data

Sweden, as well as other Nordic countries, has a long tradition of providing population statistical information covering different subjects of interest (demographic, economic, business etc.). The earliest uses of administrative data within local parishes date back to the 18th century, while modern uses began in the1940s when **personal identification numbers** (PIN) were introduced in Sweden (Figure 4) (181). To collect information on a certain unit level (e.g. marriage and divorce of citizens and residents, air pollution levels in municipalities in Stockholm), and then store, and record the information longitudinally, **registers** are generated and operated by national and regional authorities. Today there are more than 50 national registers at Statistics Sweden and the National Board of Health and Welfare covering individual health and activity data across the lifespan (182).

Ideally, register data should be as accurate as possible and with comprehensive coverage for the purpose of statistical analysis and the generalizability of findings. However, incomplete registers can still be useful for certain research purposes.

3.1.1 Personal identification numbers (PIN)

All persons who are legally registered in Sweden have been assigned a **PIN** (personnummer in Swedish) since 1947. Immigrants who become permanent residents or intend to stay in Sweden for more than 365 days are also assigned a PIN (181).

The PIN consists of a person's date of birth, a birth number and a check digit. It is a unique identifier of a person except for some rare cases. For example, a PIN can be re-used on an immigrant from another deceased person. A change of PIN can also happen when the date of birth or sex was incorrectly assigned to immigrants or newborns. Thus, in order to interlink register data and avoid the potential pitfalls due to reuses/changes of PIN, two individuals with the same PIN are assigned different random serial numbers and an individual with more than one PIN during his or her life will have only one serial number for research purposes.

3.1.2 National registers

Total Population Register

The **Total Population Register** (TPR) was established in 1968 by Statistics Sweden and has been used as a sample basis to provide information on the population and its changes (Figure 4). Today, the TPR includes more than 9 million residents who registered in Sweden at the

date of birth or immigration at the Swedish Tax Agency. Information about births, deaths, immigration and emigrations, changes in civil status and citizenship is also present in the TPR.

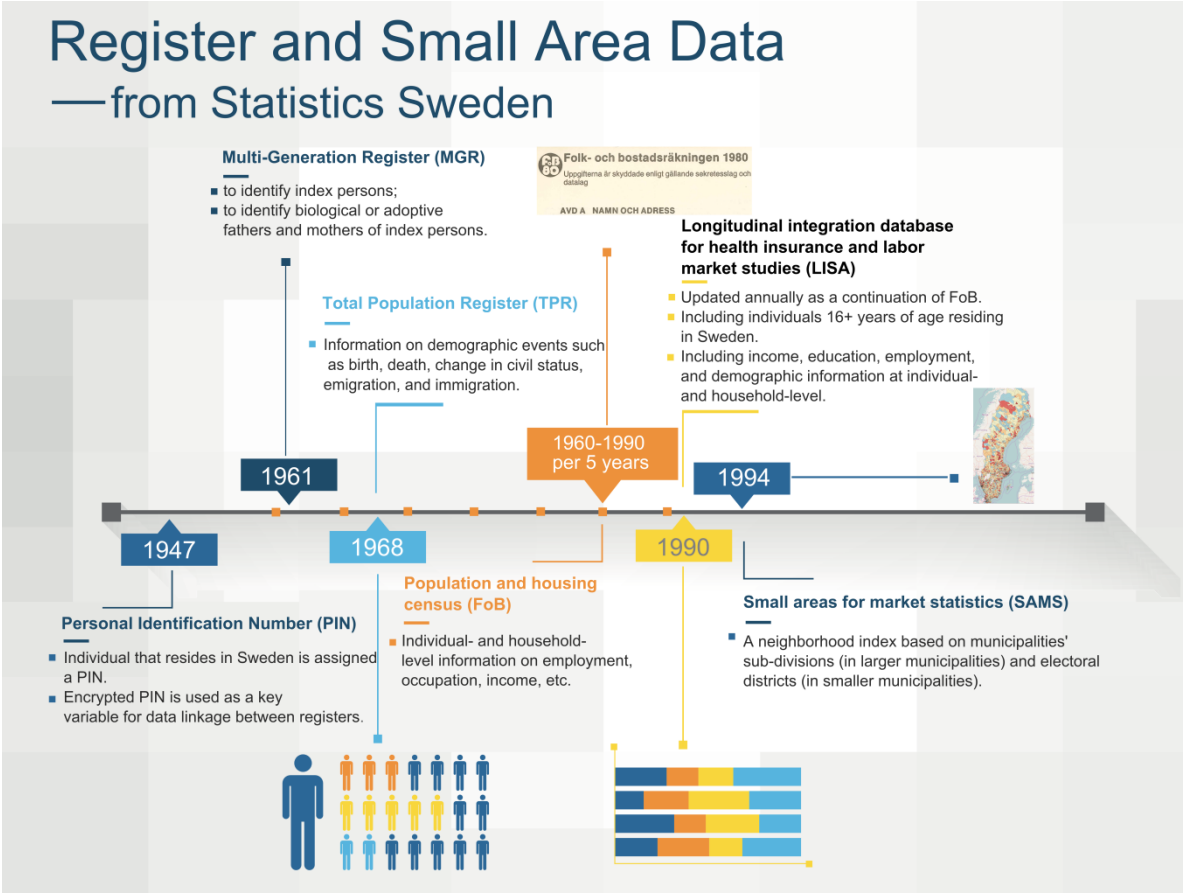


Figure 4. Register and Small Area Data from Statistics Sweden

Multi-Generation Register

The **Multi-Generation Register** (MGR) was established in 1961 by Statistics Sweden and includes information on all residents who were born in Sweden in 1932 or later or came to Sweden after 1947 (referred to as "index persons") (Figure 4). The MGR has been created on the basis of TPR data and has excellent coverage since maternal information (biological or adoptive) is available on 100% of index persons born after 1961 and paternal information is available for approximately 98% (183). The register can therefore be used to identify family members of the index persons such as parents, children, siblings, cousin, etc. With the use of PINs, other information can be retrieved via linkages with other registers.

Longitudinal integration database for health insurance and labor market studies

The **longitudinal integration database for health insurance and labor market studies** (LISA by Swedish acronym) is a register-based longitudinal database operated by Statistics Sweden (Figure 4). It includes socioeconomic information about Swedish households from 1990 onwards as an upgrade of the national population and housing censuses (1960-1990, with approximate 5-year intervals). Existing data on education, income, occupation and

employment, for all individuals above 16 years old from different registers in Statistics Sweden is integrated by linking individuals to workplaces and educational institutions on a yearly basis.

Cause-of-Death Register

The **Cause-of-Death Register** (CDR) contains information from 1961 on all deceased people who were registered in Sweden at the time of death (Figure 5). The CDR also includes data about the place of death - whether in Sweden or abroad (184). In approximately 1-2% of all deaths, the National Board of Health and Welfare could not obtain a death certificate, but death records are retained in the CDR without medical information. The underlying cause of death is coded using the International Classification of Diseases, 7th-10th revisions (ICD-7 to-10). However, stillbirths and non-residents who died in Sweden are not included in the CDR.

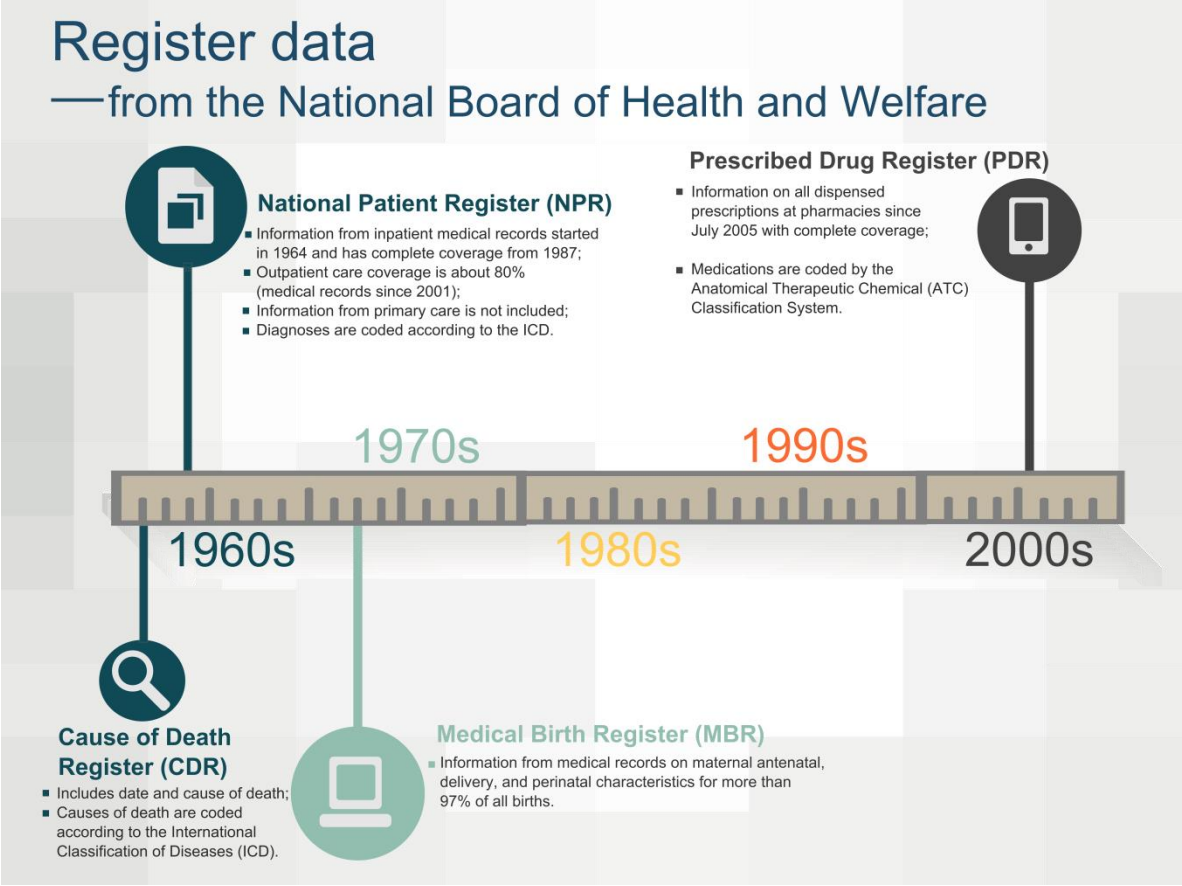


Figure 5. Register data from the National Board of Health and Welfare.

National Patient Register

The **National Patient Register** (NPR) was founded by the National Board of Health and Welfare to collect data on individuals who were hospitalized due to somatic diseases in Sweden between 1964 and1965 (Figure 5). Psychiatric inpatient care was archived from the early 1970s. Visits or day-surgeries at outpatient care clinics were included in 1997 and 2001, respectively. However, primary healthcare visits are still provided at the county council level and not yet included in the NPR.

Information on primary and secondary diagnoses, date of hospital admission and discharge from inpatient care, or date of visit from outpatient care is recorded in the NPR. Diagnoses are coded based on the Swedish adaption of ICD-7 through -10. The coverage of inpatient care has been considered to be 85% by 1983, and almost 100% by 1987; however, coverage of outpatient care is approximately 80% due to the lack of information on private clinics/hospitals (185).

Medical Birth Register

The **Medical Birth Register** (MBR), initiated in 1973 by the National Board of Health and Welfare, contains extensive information on pregnancies, deliveries and newborn (including stillbirths with more than 28 weeks of gestational age) infants in Sweden (Figure 5). A delivery record must be present in order to be included in the MBR, however medical records from antenatal and neonatal care can be missing. Thus, the quality of different variables in the MBR varies. Currently, more than 97% of all births by women residing in Sweden are included in the MBR (186).

Prescribed Drug Register

The **Prescribed Drug Register** (PDR) contains information on the dispensed medications from individual purchases at all pharmacies in Sweden since July 1, 2005 (Figure 5). All medications are coded based on the Anatomical Therapeutic Chemical (ATC) classification system. The Swedish Pharmacy service company (Apotekens Service AB) takes the responsibility of uploading all the information about the purchases (patient, medication, prescriber, and pharmacy) to the National Board of Health and Welfare each month. The register does not include the indication from the prescription to the patient, but a linkage to other registers (e.g. the NPR) via PIN can provide a possibility to explore medication and disease associations.

3.1.3 Regional registers

The **Stockholm regional health care data warehouse** (VAL by Swedish acronym) is a regional healthcare-related register including approximately 3 million residents in Stockholm County (Figure 6). Individual records from hospital visits (inpatient, outpatient, and emergency) and primary healthcare centers have been reported to Stockholm County Council for administrative and monitoring purposes since 2003. This register provides an additional source of individual diagnoses from the NPR. However, quality check of the data has not been performed (187).

The **clinical database for child and adolescent psychiatry in Stockholm County** (PASTILL by Swedish acronym) is a regional register, including information on child and adolescent psychiatric inpatient and outpatient care within Stockholm County since 2000 (Figure 6). Diagnoses are coded using the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) until 2008 and the ICD-10 since 2009 (188).

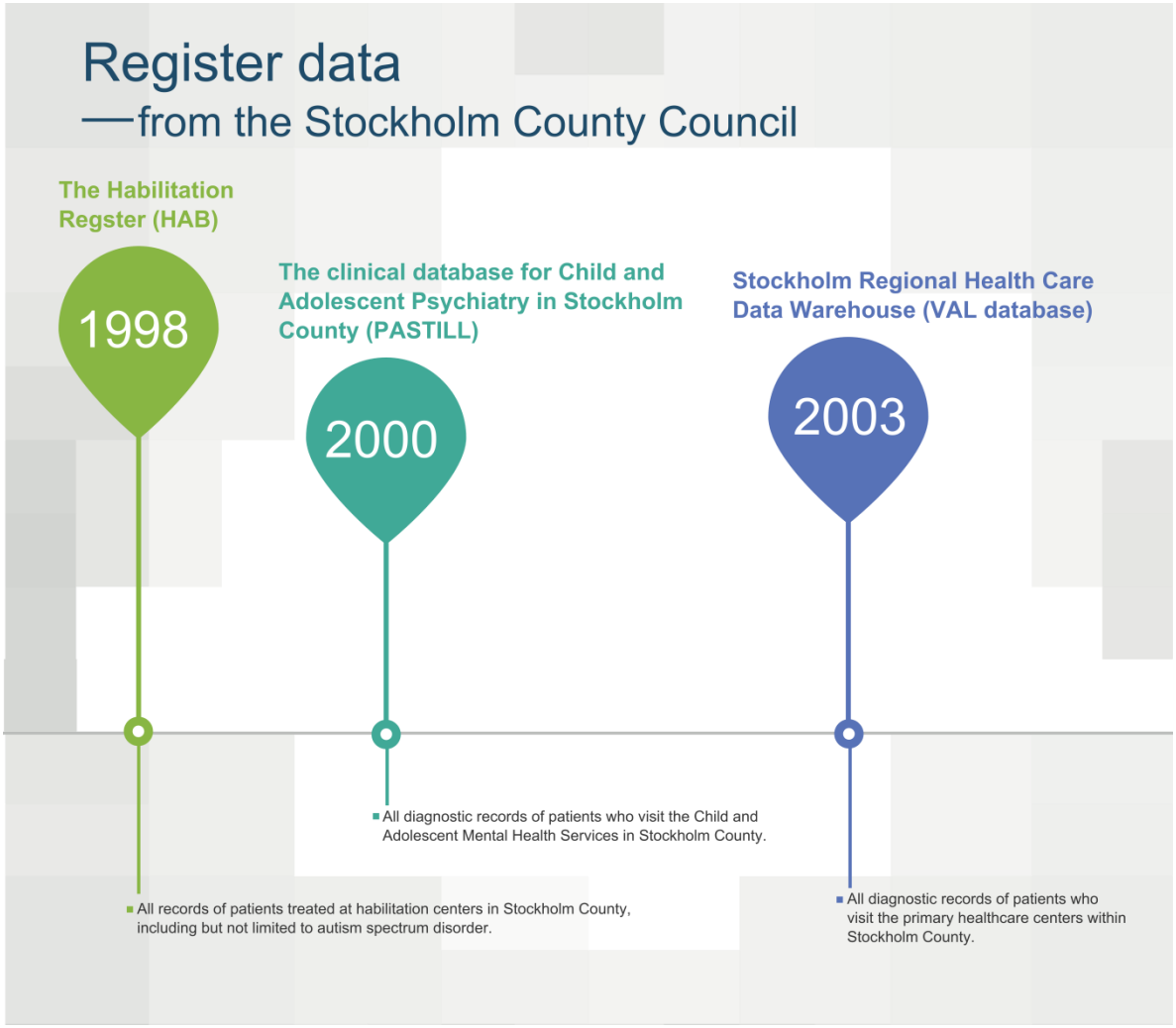


Figure 6. Register data from the Stockholm County Council

The **habilitation register** (HAB by Swedish acronym) is a regional register recording the habilitation services provided by the Stockholm County to children and adolescents with disabilities and their families since 1998 (Figure 6). Services are provided free of charge by the municipality to those with an established diagnosis such as ASD and ID.

The emission database (NO_x and PM) in Stockholm

The emission database is administrated by the Stockholm Air Quality and Noise Analysis of the Environment and Health Protection Administration in Stockholm. Since 1993, the database has been updated on a yearly basis and provides detailed information on emissions from sources including road, air and ferry traffic, residential heating, and construction dusts (189).

3.1.4 The Swedish Twin Registry

The Swedish Twin Registry was started in the1960s and consists of about 95,000 twin pairs. It contains invaluable information on twins' genetic and environmental exposures and various health outcomes collected from more than 30 research projects (190-192).The register is currently administrated by the Department of Medical Epidemiology and Biostatistics at

Karolinska Institute. Of particular interest in the current thesis is the Child and Adolescent Twin Study in Sweden (CATSS), an ongoing longitudinal twin study in the STR (193). More detailed information about CATSS is described in the method section.

3.2 General aspects on causal inference

A central question that motivates many empirical studies in medical sciences is about causality (sometimes referred as causation or causal relationship). For example, is there any efficacy of a medication in children below 5 years of age? How many asthma cases could have been avoided by reducing air pollution levels? Due to the counterfactual issue of causal inference (194), a well-designed randomized controlled trial (RCT) is considered a powerful methodology to investigate the causal relationship. RCTs provide excellent internal validity (i.e. no confounding and selection bias) and more precise efficacy measure of certain intervention under ideal conditions. However, some pitfalls of RCTs should not be ignored in terms of limited external validity, rare outcomes, costs, time, and ethical issues. For example, pregnant women and participants with comorbidities are often excluded in RCTs. Even if there is causal relationship, the effect may be limited to a subgroup or during a short follow-up period. In the case of investigating effects of air pollution exposure, researchers cannot assign random selected participants to some residential areas with either high or low pollution level.

Given the limitations of RCTs, epidemiologists can also suggest potential causal inference based on observational studies. Examples include analyses of population-based data which have shown a relationship between maternal smoking during pregnancy and childhood asthma (195), air pollution and lung cancer (196), and socioeconomic status and psychopathology (197). However, making causal inferences based on findings generated from observational data can be challenging. One challenge is that we cannot be confident the exposed group is the representative of what would have happened to the non-exposed group if they had been exposed without randomization. Therefore, important limitations of observational studies such as confounding, loss of follow-up, reverse causation, recall bias, selection and measurement bias should be discussed when interpreting observed association.

A range of methods can be applied to account for some of the issues in observational studies, but I will describe two methods in this thesis. First, the causal diagram can help to carefully identify potential confounders with a priori knowledge about the causal network of exposure, outcome, and other covariates (198). Second, the family-based quasi-experimental design may tackle some confounding factors shared within families (199).

Directed acyclic graphs (DAGs) can be used to illustrate briefly the casual diagram. In Figure 7, the research question investigating whether there is an association between maternal asthma (exposure) and offspring ASD (outcome) is presented as the blue arrow with a certain direction. A confounder, defined as a variable that is associated with exposure and outcome to a certain direction, can be measured or unmeasured. An example of an unmeasured confounder here is the genetic susceptibility for maternal asthma and offspring ASD in study

IV. Adjustment for (also referred as conditioning on or controlling for) maternal age or genetic susceptibility can close the causal path of Exposure ← Confounder → Outcome. The association can be mediated by some factors (called mediators), e.g. pregnancy and delivery complications. Adjustment for mediators can block the causal path of Exposure → Mediators → Outcome, and the direct path between Exposure and Outcome can be estimated. In some conditions, the effect of exposure on outcome may vary across strata of a third variable (called effect modifier). This can be handled by an interaction term in the statistical model.

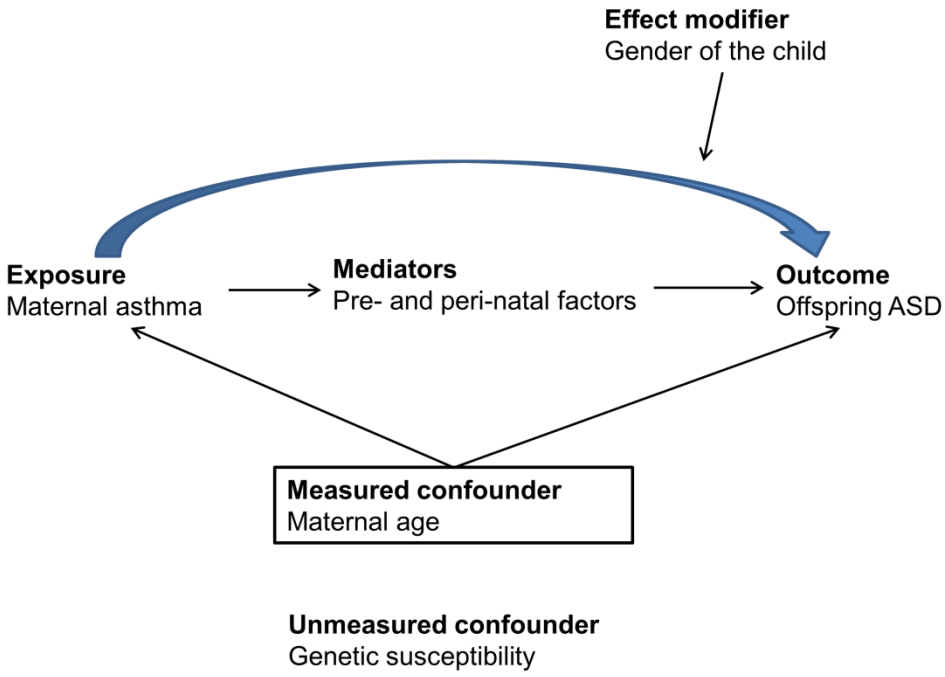


Figure 7. An example causal diagram using directed acyclic graphs.

3.3 Study I

3.3.1 Study population and measures

In this population-based cohort study, we included all children born in Sweden between April 1, 2006 and December 31, 2008 (n=288, 872) from the MBR and followed from birth to December 31, 2010. Parents' PINs were retrieved from the MGR. In order to ensure complete information on an individual's highest attained level of education, we excluded children of parents who were born abroad and migrated to Sweden after 15 years of age (n=77,352, 26.8%).

Sweden has a universal healthcare system, which aims to ensure that all citizens receive health services as needed without financial difficulty to pay for. More than 80% of total expenditure on healthcare in Sweden is funded by government (i.e. county councils, local

authorities and municipalities). Examples of public-financed healthcare services include primary healthcare, hospital inpatient care, outpatient specialized care, emergency care, patient transport support, home care, dental care for children and young adults, public health and preventive services, inpatient and outpatient prescription medications, disability support and rehabilitation services. Swedish citizens, long-term residents, adult asylum seekers, asylum-seeking and undocumented children have equal access to healthcare services through the universal healthcare system. In addition, In addition, there are two types of private healthcare services available for patients, depending on whether they are under contract with the National Healthcare Services or not. About 16 % of the total health expenditure is directly paid by patients (200), the majority of which is for medications (201). Patient fees per outpatient/emergency care visit vary from 200 to 350 SEK, and fees per primary healthcare visit and hospital stay per day are cheaper, from 80 up to 200 SEK. Additionally, there is a ceiling cost set by the National Board of Health and Social Welfare for patients to limit their expenditure on hospital visit (up to 1,100 SEK) and medications (up to 2,200 SEK) (202) .

Therefore, two common care-seeking pathways for children with asthma symptoms are via contact with primary healthcare centers, occasionally followed by referral to hospital in- or outpatient care, and via direct contact with hospital inpatient care. Two ways of measuring incident asthma were suggested accordingly:

1. From the NPR, we identified patients with asthma from hospital inpatient or outpatient care by the date of first hospital admission or outpatient care visit containing a primary diagnosis of asthma, according to the diagnostic codes (J45-J46) of ICD-10. We also estimated time to the next inpatient care visit after the first asthma diagnosis based on time from the first visit at inpatient (discharge date) or outpatient (visit date) care to the date of the next admission to in-patient care with an asthma diagnosis.

2. From the PDR, patients actively treated with asthma medications were identified. To compensate for the lack of diagnostic information from primary healthcare, incident asthma was defined based on the date of first filled prescription of ICS, β2 agonists, fixed-dose combination of ICS and β2 agonists, and LTRA (by ATC codes R03AC, R03AK, R03BA, and R03DC, respectively) if the individual has filled one more prescription of any indicated mediations within 24 months. This asthma measure has been previously validated and used in population-based observational studies (203, 204). Additionally, to calculate the average daily dose of each type of medication, we divided the total amount of active ingredients of all dispensed packages by the total number of days since the date of first asthma diagnosis.

Parental SES was measured using parental disposable income and education information from the LISA database, which is updated on a yearly basis. Disposable income includes personal income after tax deduction, study allowance, and other social assistance benefits. To make it comparable for families with different sizes and compositions, we calculated the disposable income per consumption unit by summing up individual disposable incomes, adjusting for consumption units within family, and dividing it into quintiles. Parental education was measured as the highest educational level attained between parents and

grouped as compulsory school (i.e. 9 years of education), high school (10-12 years of education), some college (13-14 years of education), and college graduate or higher education (≥15 years of education).

3.3.2 Statistical analysis

We used Cox proportional hazard models for the following binary outcomes: the first asthma diagnosis, at least two dispensed asthma medications (since the first active prescription), an inpatient asthma diagnosis, and an outpatient asthma diagnosis. A Cox model is a popular statistical model to explore the relationship of various covariates including main exposures and lifetime variables of interest of an individual through hazard function. Attained age was set as the underlying time scale. Individuals were censored at the date of death, migration, or end of the follow-up period (31 Dec 2010). Cumulative hazards for asthma by SES indicators were estimated with the Nelson–Aalen method. The proportional hazards assumption was tested based on the Schoenfeld residuals as well as by graphical examination. When the proportional hazard assumption was violated, we treated parental SES as time (age)-dependent covariates and time-dependent hazard ratios (HR) were estimated. Estimates presented in the study were adjusted for maternal age and marital status during pregnancy, child's gender, parity, healthcare regions, and metropolitan areas. Family clustering was taken into account in the models using the robust standard errors.

In the sub-cohort of children with asthma (≥1 diagnosis or ≥2 medications), we explored the associations between parental income and education at the time of first diagnosis/medication and average doses of dispensed medication. Linear regression models were used to estimate the regression coefficient exp (β) from logarithmic transformed data on daily doses together with 95% confidence intervals (CI). Among children with ≥2 asthma diagnoses, we further explored the association between time-dependent SES indicators and the risk of inpatient care visit after a first asthma diagnosis using Cox models. Time from first diagnosis was set as the underlying time scale and the level of previous hospital visit (inpatient/outpatient) was adjusted in the model.

3.4 Studies II & III

3.4.1 Study population and measures

Studies II and III were based on two samples within Stockholm County. First, all twins who were born in Stockholm County from 1992 and onwards, invited to the CATSS by November 2011, and completed the neurodevelopmental assessment at the age of 9 or 12 years old (n=3,426, response rate 68.8%) were included in Study II. Second, a sub-sample from the Stockholm Youth Cohort (SYC) was used as the study base for case and control selections in Study III. In the sub-sample, we included children born and living in Stockholm County during1993- 2007 with their biological mothers living in Stockholm County one year before and one year after the child's birth (n=277, 478). More detailed information on CATSS and the Stockholm Youth Cohort has been provided in previous publications (188, 193).

ASD and ADHD in Study II was measured by the Autism-Tics, ADHD, and other Comorbidities (A-TAC) inventory, a psychiatric symptom-based telephone interview at the 9 or 12 years of age (205). A-TAC is accessible online for free and contains 96 gate questions to screen childhood ASD, ADHD, and other targeted disorders based on DSM-IV criteria and clinical features identified in previous literature and clinical practice. Detailed information on the psychometric properties of the A-TAC is provided elsewhere (193, 206, 207). Three options of response, "No", "Yes, to some extent", and "Yes" were coded as 0, 0.5, and 1.0 accordingly for each question. We used the lower cutoff value at 4.5 as well as the higher cutoff value at 8.5 from the 17-question summed scores to indicate ASD. ADHD lower (at 6) and higher cutoff values (at 12.5) were based on the summed scores of 19 ADHD symptoms relevant questions.

Information on ASD in Study III was obtained from all ASD-related services provided by, or under contract with the Stockholm County Council. The care-seeking pathways for ASD were slightly different from those described for asthma. In Sweden, more than 99% of the children receive regular examinations for general health, growth, and developmental screening for free at pediatric primary care centers (Barnavårdscentral in Swedish) at 1, 2, 6, 10-12, 18, 30-36, 48, and 60-72 months of age. Nurses, general practitioners, and /or pediatricians, child psychiatrists, speech therapists, or parents can request for a case referral if children present with difficulties of learning, speaking, playing, or other developmental and behavioral problems. Then, a comprehensive diagnostic evaluation of suspected ASD is made by a specialist team with at least a psychologist and a medical doctor at child pediatric and mental health services. Habilitation services, as an additional care-seeking pathway for children with ASD, are provided free of charge by the municipality to children with an established diagnosis (188). To cover all sources of ASD-related care including the NPR, the VAL, the PASTILL, and the HAB, we identified 5,529 ASD cases, with or without presence of ID based on ICD and/or DSM codes (ASD: 299 in ICD-9 and DSM-IV, F84 in ICD-10 and ID: 317-319 in ICD-9 and DSM-IV, F79 in ICD-10 respectively) from Stockholm County. We selected a random sample of 20,000 children as controls from the study base, and excluded 420 who developed ASD during follow-up. Furthermore, adopted children (n=17), multiple births (n=747), births not recorded in the MBR (n=972) from selected cases and controls were also excluded.

Exposure to traffic-related air pollution was estimated using dispersion models incorporating the mother's residential address during pregnancy and the child's residential address during the first year of life for both studies. Additionally, we estimated trimester-specific pollution levels during mother's pregnancy and during child's 9th year of life in study II. Detailed descriptions of the air pollution exposure assessment are available in previous publications (140, 208). Briefly, relevant residential addresses were geocoded and pollutant levels emanating from road traffic were estimated at these coordinates from dispersion models and used to calculate annual average concentrations for NO_x and PM_{10}. To account for changes in exposure levels among those moving to another residence, time-weighted NO_x and PM_{10}

concentrations related to road traffic emissions were calculated based on all registered addresses during the pregnancy, the child's first and 9th year of life.

3.4.2 Statistical analysis

We used generalized estimating equation models in Study II to account for correlated exposure and outcome measures within twin pairs. Odds ratios (OR) and 95% CI for ASD associated with a 5th to 95th percentile rising in NO_x or PM_{10} levels were estimated in crude models and with adjustment for maternal age and smoking during pregnancy, maternal marital status parental education and income, neighborhood deprivation at the year of child birth, gender, and parity of the child (209).

To assess the effect of exposure to pollutants during mother's pregnancy and child's first year of life on ASD in Study III, we used conditional logistic regression models and conditioned on calendar year and municipality of birth. Fixed exposure increments per 20μg/m^3 for NO_x and per 10μg/m^3 for PM_{10} were used in all models to estimate the risk of ASD outcomes. To assess effect modification, we examined the association between exposure to either pollutant and subsequent ASD by maternal marital status and smoking during pregnancy, residential mobility during mother's pregnancy, parental education, and neighborhood deprivation at child birth, gender, and parity of the child via inclusion of the interaction terms in the regression models.

3.5 Study IV

3.5.1 Study population and measures

This nested case-control study was based on data linkage of several Swedish registers via unique PIN (210). Briefly, we selected a birth cohort from the MBR including all singletons born in Sweden between January 1, 1992 and December 31, 2007 (N=1,579,263) and followed them until December 31, 2013, emigration or death. Each person was linked to his/her biological mother, father, siblings and cousins through the MGR.

Inclusion criteria for cases were a primary or secondary diagnosis of ASD from NPR since birth. To differentiate between individuals with high and low functioning ASD, we further retrieved cases' diagnoses on ID during the follow-up. ASD and ID definitions were consistent with those used in previous studies (188, 211).

Using an incidence density sampling method, we first selected 10 biologically unrelated controls for each case. Second, we selected four types of family members of each case with different degrees of genetic relatedness: full-siblings, half-siblings, full-cousins, and half-cousins from the MGR. All full-siblings born in 1995 or later were eligible as controls. Half-siblings, full-cousins, and half-cousins were eligible as controls if having the same gender, less than 5 years of age difference, being singleton, alive, and ASD-free at the age when the case received ASD diagnosis. In case where multiple eligible controls were identified from the same extended family, we randomly selected one control for each degree of relatedness.

To retrieve information on parental asthma for cases and controls, we used records from any of the three registers, i.e. MBR, NPR, and the PDR. In the MBR, a tick-box for asthma/lung diseases ever for the mother was indicated at her first antenatal visit from 1992 onwards. In the NPR, we used any primary diagnosis of asthma from outpatient visits since 2001 or hospitalization records since 1961. In the PDR, dispenses of asthma medications including ICS, LTRA, fixed-dose β2-ICS combinations and β2-agonists from July 2005 have been previously validated (204) and used as a proxy of asthma diagnosis. Parental asthma was categorized as asthma from either parent, maternal asthma, or paternal asthma for analysis.

Information about asthma medication use during pregnancy was retrieved from two sources, i.e. midwife-reported medication use during pregnancy in the MBR since 1995 and all dispensed medications from pharmacies recorded in the PDR since July 2005. Two forms of β2-agonists were particularly addressed in the study, as systemic β2-agonists (i.e. oral and injection) used to be mainly administered to suppress premature labor, while inhaled β2-agonists were primarily indicated for asthma. We categorized the exposure to asthma, β2-agonists and other asthma medications into four groups: with asthma but no medications, systemic β2-agonists only, inhaled β2-agonists with or without other asthma medications, and other asthma medications without any β2-agonists.

3.5.2 Statistical analysis

We conducted conditional logistic regression analyses to estimate OR with 95% CIs of ASD by parental asthma and asthma medication use during pregnancy. In addition to crude models, we also provided estimates adjusted for maternal smoking, BMI, and marital status during pregnancy, parents' countries of birth, parental age and education at child birth, as well as the parity of the child. First, the association between parental asthma and offspring ASD overall, with and without ID was estimated using cases and unrelated controls. Second, we performed separate analyses using cases and their half-sibling, full-cousin and half-cousin controls. This allowed us to account for unmeasured familial confounding factors shared by cases and their relatives as well as measured confounders and mediators mentioned above. Full-sibling controls sharing the same parents with cases were not included here. Third, to investigate the association between prenatal exposures to β2-agonists, and other asthma medications during pregnancy with subsequent ASD, we restricted the sample to children born to mothers with asthma from 1995 due to medication data availability. ASD cases were compared to unrelated and sibling controls, with not exposed to any asthma medications as the reference group.

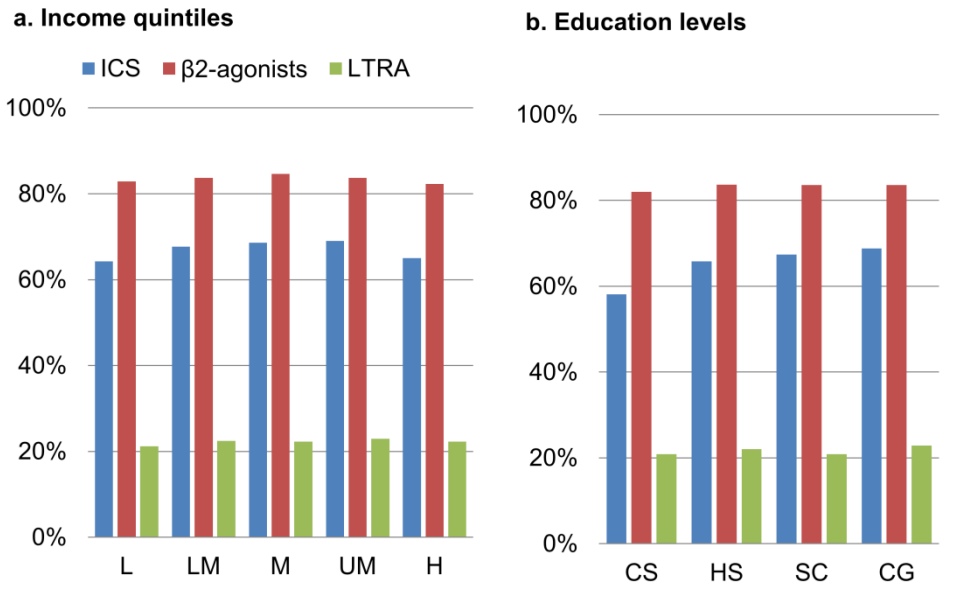
4 Main results and interpretations

4.1 Study I

4.1.1 Results

In a birth cohort of 211,520 children, we found a consistently positive association for parental education and a time-dependent association for parental income with preschool asthma. Children of parents with lower education were at higher risk of asthma (measured by ≥ 1 diagnosis or ≥ 2 medications) regardless of age. In contrast, children of parents with the lowest compared to the highest income were at higher risk of asthma during the first year of life, but lower risk after the first year of life (Figure 1 in paper 1).

Among 13,990 children diagnosed with asthma within the birth cohort, less than 70% had at least one package of ICS dispensed and 21-23% had LTRA dispensed (Figure 8). No difference in average dose of dispensed controller medications by parental income was observed. However, the average doses were significantly lower among children of parents with lower education compared to those of parents with highest education (Figure 4 in paper 1).



Abbreviations: L=Lowest, LM=Lower-middle, M=Middle, UM=Upper-middle, H=Highest income, CS=Compulsory school, HS=High school, SC=Some college, CG= College graduates or higher.

Figure 8. Frequency of medication consumption among children diagnosed with asthma from different SES groups.

4.1.2 Interpretation

Most results were consistent with prior literature on parental education and the risk of childhood asthma. For example, a cross-sectional study from Denmark and two studies from the Netherlands demonstrated a similarly increased risk (23%-53%) of asthma associated with lower parental education levels (48, 212, 213). Several pathways including exposure to household allergens, traffic-related air pollution, tobacco smoke, maternal stress, as well as maternal obesity may partially explain the association (214-217). However, the temporal effect of parental income on preschool asthma is much less described. The Generation R study reported a time-varying effect on wheezing symptoms by parental income but in quite the opposite direction from what we observed. They reported a positive association of parental income and preschool wheezing at age one and a negative association at age 3-4 (213). The discrepant findings could possibly be explained by their self-reported parental income measures at two years of age, wheezing symptoms rather than asthma diagnosis, and different covariate adjustment. For example, breastfeeding behavior, which is a time-dependent covariate, was adjusted for in their study but not in ours (213). The prevalence of transient wheezing also peaks before three years of age, which could possibly explain the increased risk seen in their study (218).

Furthermore, our finding that children with lower parental education have lower controller medication (i.e. ICS and LTRA) dispensing rates was consistent with previous findings (219). Therefore, we speculate that children of parents with lower education may not be sufficiently surveilled and followed up after initiating the use of controller medications (220).

4.2 Studies II & III

4.2.1 Results

The distribution of NO_x and PM_{10} levels during pregnancy, child's first, and the 9^{th} years of life are presented in Table 3 using samples from the CATSS and the SYC. We observed decreases of 10-15% in average levels of NO_x between pregnancy and the child's first year of life in both studies. Furthermore, average NO_x levels dropped from 12.69 to 5.40 $\mu g/m^3$ during a10-year observation period for CATSS. On the other hand, average levels of PM_{10} remained relatively constant over the study period in both studies.

We did not find any evidence of an association between NO_x and PM_{10} levels during the prenatal and postnatal period and subsequent ASD and ADHD symptoms in CATSS, or with ASD clinical diagnosis in SYC (Figure 9). Furthermore, no difference was observed based on low and high cutoff values of ASD and ADHD symptoms, or the comorbidity of ASD with ID. Among children from SYC, a significant interaction of pollutant levels with neighborhood deprivation and residential mobility was observed (Table 4). There was a suggested inverse relationship between exposure to NO_x and PM_{10} with ASD overall for those in the most deprived neighborhoods. A decreased odds for ASD overall with exposure to NO_x and PM_{10} pre- and postnatally was seen among those whose mothers changed their

residential address during pregnancy (p values for all interactions<0.03). However, these interaction effects were not observed for ASD with ID.

Table 3. Distribution of estimated average NO_x and PM_{10} levels based on samples from CATSS and SYC.

		Mean, SD	5^{th} - 95^{th} percentile	Median
CATSS (n=3,426)				
Pregnancy	NO_x	12.69±10.94	1.49-34.53	9.00
	PM_{10}	4.19±3.10	0.58-9.38	3.34
First year of life	NO_x	10.78±9.49	1.26-30.59	7.60
	PM_{10}	3.85±2.90	0.54-9.04	3.06
Ninth year of life	NO_x	5.40±4.83	0.70-15.75	3.98
	PM_{10}	3.26±2.63	0.50-8.23	2.58
SYC (n=23,373)				
Pregnancy	NO_x	11.04±11.39	1.23-30.27	7.80
	PM_{10}	4.38±3.22	0.60-9.98	3.65
First year of life	NO_x	9.83±10.33	1.09-27.10	6.93
	PM_{10}	4.21±3.14	0.60-9.79	3.48

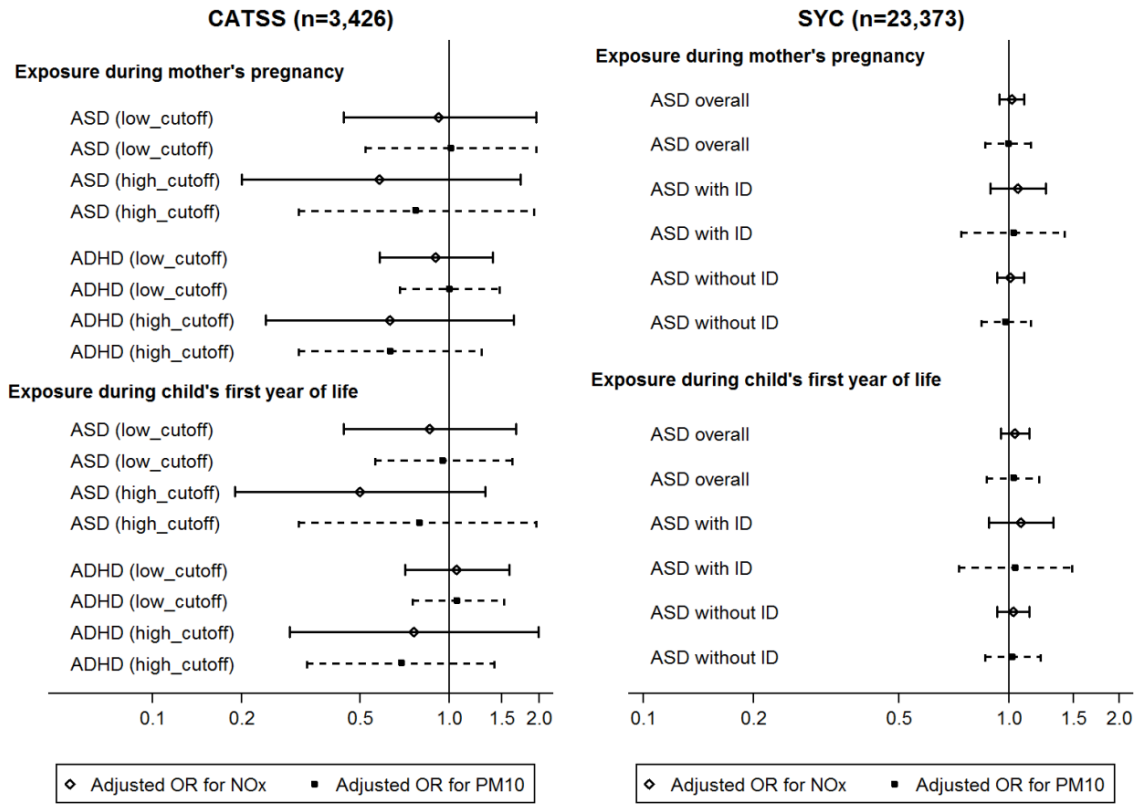


Figure 9. ASD risk by NO_x and PM_{10} levels during prenatal and postnatal period.

Table 4. Association between NO_x and PM_{10} levels during pre- and post-natal period and risk of ASD stratified by neighborhood deprivation and residential mobility in the SYC cohort. Models were conditioned on birth year and municipality and adjusted for maternal marital status during pregnancy, parental countries of birth, parental age, income, education, employment, and neighborhood deprivation at child birth, gender, birth month, and birth order of the child.

		NO_x levels		PM_{10} levels	
		ASD overall	ASD with ID	ASD overall	ASD with ID
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Overall	Pregnancy	0.91 (0.86, 0.97)	1.06 (0.89, 1.26)	0.86 (0.77, 0.95)	1.03 (0.74, 1.42)
	First year of life	0.90 (0.85, 0.97)	1.08 (0.88, 1.32)	0.86 (0.77, 0.96)	1.04 (0.73, 1.49)
Stratified by neighborhood deprivation at the year of child birth					
Least deprived neighborhood	Pregnancy				
	First year of life	0.97 (0.87, 1.08)	0.99 (0.73, 1.33)	0.96 (0.80, 1.16)	0.94 (0.55, 1.62)
Intermediate deprived neighborhood	Pregnancy	0.97 (0.86, 1.10)	1.09 (0.77, 1.55)	0.97 (0.80, 1.18)	1.12 (0.62, 2.02)
	First year of life	0.93 (0.86, 1.00)	1.03 (0.82, 1.28)	0.86 (0.73, 1.00)	1.02 (0.65, 1.58)
Most deprived neighborhood	Pregnancy	0.93 (0.85, 1.01)	1.08 (0.86, 1.36)	0.86 (0.74, 1.01)	1.07 (0.68, 1.70)
	First year of life	0.80 (0.67, 0.95)	1.31 (0.94, 1.83)	0.81 (0.64, 1.03)	1.21 (0.74, 1.99)
		0.77 (0.63, 0.94)	1.16 (0.75, 1.81)	0.82 (0.65, 1.05)	1.06 (0.62, 1.82)
Stratified by residential mobility during mother's pregnancy					
Non-movers	Pregnancy	0.94 (0.88, 1.00)	1.08 (0.90, 1.30)	0.93 (0.83, 1.04)	1.05 (0.74, 1.48)
	First year of life	0.96 (0.89, 1.03)	1.14 (0.92, 1.41)	0.94 (0.84, 1.06)	1.08 (0.75, 1.57)
Movers*	Pregnancy	0.79 (0.68, 0.91)	0.97 (0.70, 1.34)	0.57 (0.44, 0.74)	0.95 (0.51, 1.77)
	First year of life	0.71 (0.60, 0.83)	0.79 (0.50, 1.23)	0.60 (0.47, 0.78)	0.85 (0.44, 1.62)

*Movers were defined by the frequency of the mother changing her residential address during pregnancy.

4.2.2 Interpretation

We acknowledge that NO_x and PM_{10} levels were relatively low in Stockholm County, compared to other study settings which included Taiwan, California and other U.S. states (123, 124, 126, 127, 144). The substantial reduction of NO_x levels is mostly attributed to the increased use of catalytic converters on vehicles. The smaller reduction of PM_{10} levels observed in both studies may be due to the largely constant background concentrations generated from long-distance transport and relatively small variations on concentrations generated from local traffic (221).

In contrast to the growing body of literature from the U.S. showing a positive relationship between air pollution and NDDs (123, 124, 126, 127), no clear association was observed in data from Stockholm County nor other European cohorts (i.e. three cohorts from Spain, one from Italy and one from the Netherlands) (125). Null results remain in the analyses even after adjusting for community and family SES-related factors including parental income and education, maternal marital status and country of birth as well as in subgroup-specific analyses. However, residual confounding by parental psychiatric health, moving patterns, and unmeasured indicators for SES during pregnancy and early infancy and the relatively low proportion of ASD cases with ID may still have contributed to the null findings.

4.3 STUDY IV

4.3.1 Results

We found a slightly increased risk of offspring ASD with maternal asthma and a weaker, moderate, but significantly increased risk with paternal asthma when comparing cases and unrelated controls from the selected birth cohort. In further comparisons between cases and their half-siblings, full-cousins, and half-cousins, the association between maternal, but not paternal, asthma and offspring ASD remained (Figure 10).

To further explore whether the association of maternal asthma and offspring ASD was mediated through asthma medication use during pregnancy, we used the sub-sample of children born to mothers with asthma or mothers who used asthma medications. We observed no association between prenatal exposure to any indicated asthma medications or systemic β2-agonists with offspring ASD when comparing cases with unrelated controls, and with full-siblings (Figure 11).

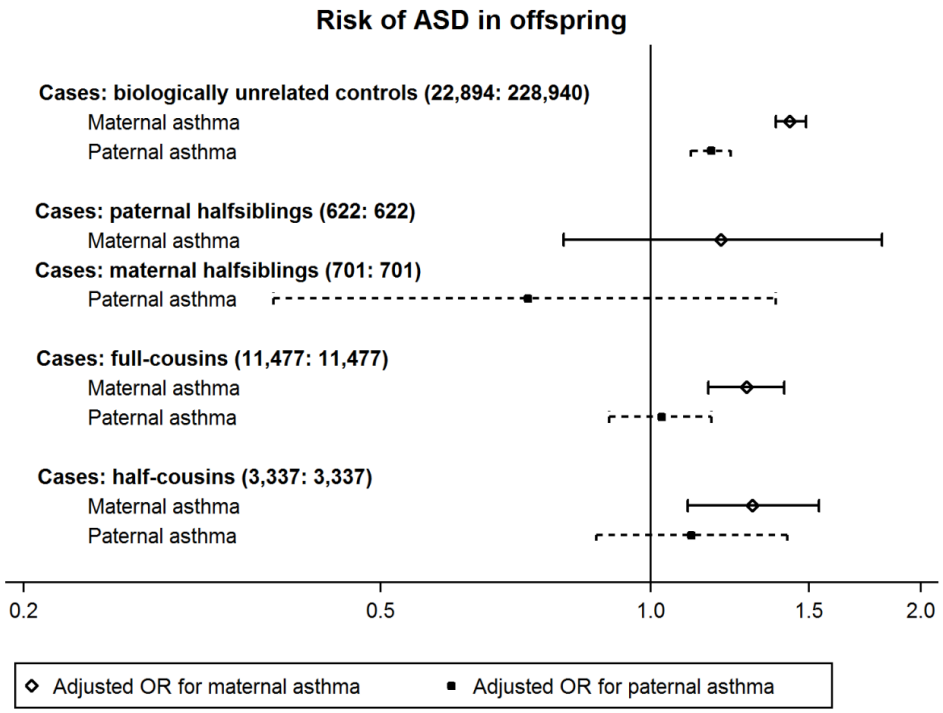


Figure 10. Adjusted ORs of ASD in offspring by maternal and paternal asthma.

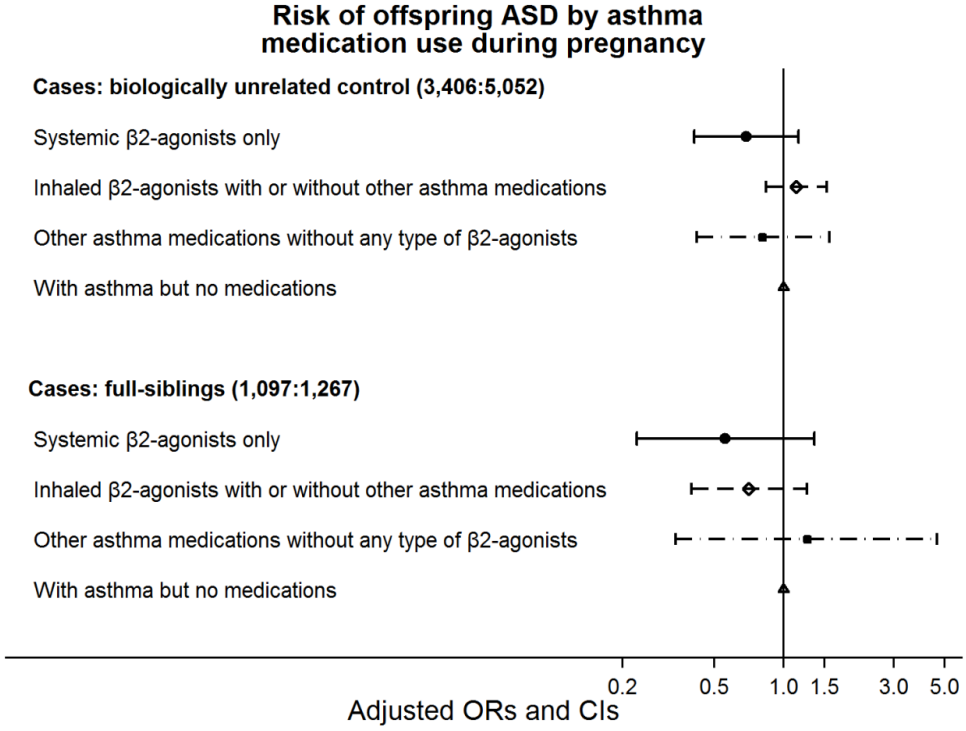


Figure 11. Adjusted ORs of ASD in offspring by asthma medication during pregnancy

4.3.2 Interpretation

Our findings on maternal asthma and offspring ASD were mostly in line with findings from a case-control study from North California, the U.S. and a cohort study from Australia (117, 118), but not with those of the recent case-control study from California (i.e. the CHARGE study) {Lyall, 2014 #106;Hertz-Picciotto, 2006 #353}. First, characteristics of cases in studies might explain the discrepancy. In the original CHARGE study, cases with full syndrome autism, but not spectrum disorders, were identified through the California Department of Developmental Services and asked to be participants {Lyall, 2014 #106;Hertz-Picciotto, 2006 #353} whereas we and the other two studies ascertained all ASD and ID cases from the national or regional registers (117, 118). Second, the average age at the end of follow-up was 10 or 15 years in the current study and the Australian study (118). Study participants in both case-control studies from California were on average below 5 years of age at enrollment (115, 117). Controls, who are still at risk of developing full syndrome autism but not followed, might also contribute to discrepancy. Third, selection criteria of eligible controls from the source population were different in the two case-control studies from California, respectively. Being ASD free was the only criteria in the North California study and the current study (117). In the CHARGE study, controls were not allowed to have any early learning difficulties or other behavioral symptoms (115).

Associations between paternal asthma and offspring ASD were less reported previously and with limited statistical power (130, 156). The smaller effect of paternal asthma and further dilution of the association in half-sibling and cousin analyses suggests the effect of paternal asthma can be confounded by familial factors.

Two previous human studies from the U.S. reported a positive association on continuous treatment with terbutaline (a β2-agonist) in utero and risk of subsequent ASD (122, 170). One study suggested that the association between maternal asthma and offspring ASD was not explained by β2-agonists treatment during pregnancy(122), suggesting a possibility of confounding by indication. The results from the second study should be interpreted with caution due to limited number of observations, characteristics of the study population, and the indication and duration of β2-agonist treatment (170). Therefore, firm conclusions concerning the safety of β2-agonists during pregnancy cannot be drawn based on current evidence. Furthermore, previous studies did not support any increased risk of NDDs in children exposed to ICS in utero (222, 223), which is in line with our findings. We therefore do not consider the mechanism linking maternal asthma and offspring ASD to be through asthma medication use during pregnancy.

5 GENERAL DISCUSSION

This thesis comprises four observational studies on risk factors for asthma and ASD in children using different study designs. The current chapter provides some important considerations on the internal (i.e. design, measurement errors, and confounding) and external validity (generalizability) of the studies.

5.1 Study designs

Although randomized experiments are often considered the gold standard in estimating causal effect and avoiding bias, they may be infeasible in some conditions due to economical, ethical, or time (e.g. long-term outcomes) constraints. Therefore, the Swedish registers provide rich data sources to investigate important research questions and can be used in different observational study designs. Furthermore, no recruitment process is needed comparing to average cohort studies or RCT, eliminating the potential risks of selection and drop-out bias.

The cohort design can be either prospective or retrospective. In a prospective cohort like study I, more than two groups of individuals are categorized based on their parental SES, followed over time, and compared for the incidence of asthma. In retrospective cohorts (e.g. study II), information on outcomes has been recorded in the registers and recall bias can be eliminated. The case-control designs, as in study III allow rare outcomes such as ASD to be studied. Two groups of people with and without ASD are sampled from the general population or specific sub-population and compared for the occurrence of exposure. The nested case-control study (e.g. study IV) is simply designed as a case-control study nested within a population cohort. Unlike a classical case-control design, this sampling method involves selection of controls from a dynamic population, which allows time-varying incidence of the cases. In addition, the odds ratio estimates may be closer to the hazard ratio estimates from cohort analysis (224).

The cohort, case-control, and nested case-control designs, however, are still subject to potential biases due to some residual or unmeasured confounding factors. Therefore, family-based designs such as study IV, take advantage of sub-samples from a larger population in a more informative way and account for some genetic and/or environmental confounding in analyses. For instance, comparing cases with full-siblings who share on average 50% of the segregating genes can help rule out some potential genetic confounding effect based on the observed association at population level. However, it should be noted that such designs are based on specific assumptions which could cause limitations in the interpretation of the finding and generalizability. For example, the sibling- and cousin-control designs assume the carry-over effect (i.e. the possibility of being exposed to asthma medications in the older sibling can influence the possibility of being exposed again and the ASD outcome in the younger sibling) (225).

5.2 Random and systematic error

Measurement error is an inevitable issue in epidemiological studies. Random errors are usually a result of chance and affect the precision of the risk estimation depending on study sample size. It is important to note that the 95% confidence interval estimates include a 5% chance of random error and are estimated without accounting for known and unknown systematic errors. Systematic errors include deviations of the results that are not introduced by chance, but by a repeated inaccurate measurement of either the outcomes or exposures, or perhaps a covariate. The internal validity of an observed association in a study is often affected by systematic error. Three common sources of systematic error are selection bias, information bias, and confounding.

5.2.1 Selection bias

In studies I and IV, asthma and ASD were defined by at least one relevant diagnosis from the NPR, which includes all inpatient and approximately 80% of the outpatient records. The narrow definitions of asthma and ASD did not include cases diagnosed at primary healthcare centers. Outcomes could therefore have been underestimated if case ascertainment did not embrace all asthma and ASD cases, especially the ones with milder symptoms. If such bias was present, the risk estimate would be biased toward the null. However, the measurement of asthma by at least two relevant medications from the PDR without a confirmed diagnosis from the NPR in study I can be viewed as an evaluation of such bias in the absence of primary healthcare data. In study II, we included Stockholm-born twins whose parents participated in the CATSS, representing 70% of the population from the original sample. The response rate probably affected the precision of association estimates and pushed the association towards null. The difference in response rates between respondents and non-respondents is a limitation for the accurate ascertainment of ASD and ADHD cases, therefore creating potential selection bias. In studies III and IV, bias on ASD case ascertainment was minimized by combining data sources of ASD-related healthcare services within Stockholm County and by comparing cases with their family members.

5.2.2 Information bias

Information bias is often a measurement error (called misclassification) of an exposure or disease outcome during the process of data collection, due to inaccurate information recall, use of sub-optimal measurement methods, or other reasons. Misclassification can be differential and non-differential depending on whether the misclassification of the exposure is related to the outcome, or vice versa.

In a previous validation study, our colleagues showed that 26% of preschool children with asthma medications had a diagnosis of bronchitis/bronchiolitis rather than asthma (204). Thus, asthma should be defined in a more rigorous way by including at least one diagnosis of asthma and at least two dispensed medications in children of this age group. This indicates that misclassification of asthma using either diagnosis or medications in study I may be present and lead to a potential underestimation of a true association.

ASD and ADHD definitions in studies II and III have been tested and confirmed with high sensitivity and specificity in a previous validation study (188, 205). However, the measurement of traffic-related air pollution levels based on residential address in studies II and III is likely to induce some misclassification bias on the exposure levels for NO_x and PM_{10}. In study III, nearly 20% of mothers or young parents changed their addresses during pregnancy and child's early life and were accordingly exposed to different levels of air pollution. When reporting the subgroup-specific associations, we observed an inverse association for air pollution and subsequent ASD among movers. This has to be interpreted with caution as moving patterns might have influenced the classification of some confounders (e.g. municipality). Furthermore, misclassification of ASD outcomes may be present due to the loss of follow-up among some older children who migrated from Stockholm County after being enrolled in the cohort. However, consistent results were observed in a subgroup of children born after 2003. In study IV, the asthma/lung disease tick-box from the MBR was used as one source of maternal asthma measurement. Misclassification bias might have been introduced by including other lung diseases such as chronic obstructive pulmonary disease and pneumonia, although they are less common in young adulthood. In the case of such misclassification, it should be non-differential and could push estimates towards the null as the mothers would not have chance to report their lung disease differently in relation to later offspring ASD. However, the sensitivity analysis confirmed that the potentially less accurate measures of maternal asthma based on MBR tick-box information did not change the estimates.

5.2.3 Confounding

Acknowledging and adjusting for confounding is crucial for reported associations in epidemiological studies (226). In the presence of confounding, the estimate of an association between an exposure and a disease outcome can be inaccurate or even spurious without proper adjustment for confounders. As demonstrated in the method section, causal diagrams have been widely applied to identify potential confounders a priori for the statistical modelling. However, unmeasured or residual confounding can still be present in each study. For example, the genetic confounding for the association between parental SES and preschool asthma in offspring was not taken into consideration in study I. In studies II and III, although SES confounders including parental income, education, and maternal marital status have been adjusted for in the analysis, residual confounding still seemed to exist in the observed inverse association among movers as well as those from the most deprived neighborhood. In study IV, as illustrated in Figure 7, the potential bias generated from unmeasured familial (genetic and shared environment) confounding has been noted. The analysis comparing cases with unrelated controls indicated that paternal asthma is associated with a weak but significant increased risk of offspring asthma. However, the association disappeared when comparing cases with sibling and cousin controls, suggesting most of the risk from the paternal side is instead attributable to familial confounding.

5.3 Generalizability

Generalizability refers to the relevance of findings based on a source population to other populations at other places (226). Although the population and characteristics, inclusion and exclusion criteria, as well as lengths of follow-up vary for each study in the thesis, the results in each study are overall generalizable at the population level since the original large samples are quite representative of the Swedish population. Specifically, the null associations of air pollution and ASD have been replicated in twins and singletons living in Stockholm, as well as children of similar age in other European cohorts (125).

5.4 Ethical consideration

According to the Swedish ethical review act (SFS no 2003:460), all studies with the intention to interact with research participants physically or mentally must be reviewed by an ethical board before initiation of the project. The participants in register-based research are involved in a different way, as their information is not directly collected by researchers but by third parties, such as governmental agencies. This also means sensitive information can be gathered and analyzed by researchers without notifying participants. To protect the integrity of personal data, ethical approval is needed for all epidemiological research to clarify the potential value of generated knowledge and minimize the risk of breaching the data integrity. The data should be used for the specific research purpose and there should be no direct contact with participants. Researchers should also manage other potential risks generated from combining too detailed information on participants (227). However, in the case that researchers need to contact and recruit specific sub-population (e.g. twins) for additional data, informed consent is required. If participants are younger than 15 years of age, both children and their parents should be informed prior to participation and give the consent.

All studies included in this thesis have been approved by the regional Ethical Review Board in Stockholm. None of the studies directly involved with study participants. More specifically, studies I, III, and IV used large population-based register data, meaning that informed consent was not required. To protect the personal integrity of all participants, the PINs are encrypted and replaced by random sequence numbers. For study II, informed consents were previously collected from both parents and children according to the Declaration of Helsinki.

5.5 Concluding remarks

The population-based and genetically informed designs allowed me to further understand the risk factors of asthma and ASD in children, expanding upon rather than replicating the findings from prior research. In particular, an age-dependent effect on offspring asthma by parental income is observed, a consistently increased risk of incident asthma, and reduced use of controller medications among children of parents with lower education (**Study I**). There seems not to be an association between traffic-related air pollution exposure during fetal and early life and subsequent ASD (**Studies II & III**). Furthermore, maternal asthma is associated with increased risk of offspring ASD, which is neither confounded by familial

factors shared in siblings and cousins, nor mediated by use of asthma medication during pregnancy (**Study IV)**.

6 Postscript

6.1 Funding sources

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6.2 Future perspectives

In study I, we have addressed the association between parental SES and preschool asthma using a population-based cohort design. From the etiological perspective, others have reported that childhood asthma is affected by environmental and perinatal factors including smoking during pregnancy, fetal growth, and infections, which possibly act as mediators of the observed associations (22, 195, 215, 228). One way to address the question further is to explore the possible pathways through such variables. Another way is to explore the effect of other measures of SES including the neighborhood deprivation, occupation, and employment dynamics, as the current study only takes into consideration parental income and education. A third way to further investigate the association could be to include other asthma phenotypes as outcomes including later-onset, transient, and persistent asthma cases and the comorbidity with other allergic diseases. From the clinical perspective, the observed patterns of dispensed controller medication by parental education among diagnosed children need to be investigated for further explanations. Improved understanding of their phenotypes, asthma severity, comorbidity, surveillance and follow-up of parental asthma-related knowledge is needed for a better clinical management.

Ambient air pollution, specifically traffic-related air pollution, is a global public health issue (136, 138). Policy decisions regarding air pollution and health should be made with deliberate consideration based on evidence from specific scientific research studies. The evidence of an increased risk of ASD by elevated NO_x and PM_{10} levels is not obviously seen within Stockholm County. Careful adjustment for a constellation of confounding factors including SES, genetic susceptibility, and area related factors such as population density, deprivation, noise should be encouraged in future studies. Population-based case-control studies and within-family designs will be useful tools to further investigate other neurodevelopmental effects by exposure to air pollution. Additionally, based on the evidence on pathological changes in central nervous system caused by chronic air pollution from human, animal and cell samples, the long-term effect of NO_x and PM_{10} as well as other air pollution components' effect (e.g. ozone, $PM_{2.5}$ and $PM_{0.1}$) can be of interest (229).

Up to 45% of women with asthma experience symptom exacerbations during pregnancy (230). This subgroup of patients is also at increased risk of adverse obstetric and birth outcomes, as well as other long-term health outcomes including ASD in offspring. The main findings of study IV suggest a potential causal association between maternal asthma and offspring ASD. However, the biological mechanisms remain unclear. Furthermore, a substantial proportion of women are still under suboptimal treatment despite the existence of clinical guideline on how to manage asthma during pregnancy (231). It is therefore worth investigating whether there is a dose-response relationship between asthma severity and management with the risk of subsequent ASD. Last but not least, more studies are needed on the safety of asthma medication use during pregnancy particularly intervention studies of high quality to improve asthma management during pregnancy.

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